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Monoclonal Antibody 425 Inhibits Growth Stimulation of Carcinoma Cells by Exogenous EGF and Tumor-Derived EGF/TGF-α

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Carcinoma cells frequently coexpress transforming growth factor (TGF)-α and its receptor, the epidermal growth factor (EGF) receptor, implicating an autocrine function of carcinoma-derived TGF-α. Using a monoclonal antibody (425) to the EGF-receptor, we investigated the role of exogenous and tumor cell-derived EGF/TGF-α mitogenic activities in proliferation of cell lines derived from solid tumors. Monoclonal antibody 425 was chosen for these studies because it inhibits binding of EGF/TGF-α to the EGF-receptor and effectively blocks activation of the EGF-receptor by EGF/TGF-α. Seven malignant cell lines originating from carcinomas of colon, pancreas, breast, squamous epithelia, and bladder expressed surface EGF-receptor and secreted EGF/TGF-α-like mitogenic activities into their tissue culture media. All cell lines were maintained in a defined medium free of exogenous EGF/TGF-α. EGF and TGF-α added to the culture medium stimulated proliferation of five cell lines to comparable levels. EGF/TGF-α-dependent proliferation was significantly reduced by addition of MAb 425 to culture media. In addition, monoclonal antibody 425 reduced proliferation of the five EGF/TGF-α responsive cell lines in the absence of exogenous EGF/TGF-α. Antiproliferative effects induced by monoclonal antibody 425 were reversible and could be overcome by addition of EGF to culture media. Our results indicate that tumor-derived EGF-receptor-reactive mitogens can promote proliferation of carcinoma cells in an autocrine fashion.

Key words: carcinoma, autocrine stimulation, EGF-receptor, transforming growth factor, autocrine function

In recent years monoclonal antibodies (MAbs) have attracted considerable interest as potential immunotherapeutic agents [1,2]. MAbs reacting with growth

Abbreviations used: CRC, colorectal carcinoma; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; FCS, fetal calf serum; MAb, monoclonal antibody; TGF, transforming growth factor.

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factor receptors may have additional inhibitory effects on tumor cell growth if they block access of growth-stimulatory activities to their cognate receptors. A blocking antibody to the type I insulin-like growth factor receptor [3] has been used to demonstrate mitogenic effects of insulin-like growth factors on pancreatic carcinoma [4], neuroblastoma [5], and melanoma cells [6]. Alternatively, anti-receptor antibodies may activate growth factor receptors and thus mimic the physiological effects of growth factors. This mode of action has been described for several MAbs reacting with the epidermal growth factor (EGF) receptor [7–9]. We have described MAb 425, which has an EGF-antagonistic mode of action [10]. It recognizes a protein epitope close to the EGF binding site on the extracellular domain of the EGF-receptor, blocks binding of EGF to the high- and low-affinity EGF-receptor expressed on A 431 membranes, and effectively inhibits EGF-dependent proliferation of human diploid fibroblasts.

Transforming growth factor (TGF)-α is a structural homologue of EGF [11,12] and competes with EGF for binding to the EGF-receptor [13,14]. TGF-α is frequently expressed and secreted by human carcinoma cells in vitro and in situ [15–19]. It has been speculated that carcinoma-derived TGF-α may contribute to the deregulation of malignant cell growth by activating EGF-receptors expressed on the surface of tumor cells. This suggestion was supported by the finding that TGF-α expression by carcinoma cells is frequently accompanied by the expression of EGF-receptors [20,21].

In this study we demonstrate that MAb 425 inhibits binding of TGF-α to the EGF-receptor and significantly reduces the growth stimulation elicited by exogenous EGF and TGF-α of EGF-receptor expressing carcinoma cells. In addition, we present results indicating that tumor-derived EGF/TGF-α stimulates proliferation of EGF/TGF-α responsive tumor cells.

MATERIALS AND METHODS

Cell Lines, Reagents, and Cell Culture

Human colorectal carcinoma (CRC) cell lines SW1116, SW948, pancreatic carcinoma cell line Capan-2, breast carcinoma cell line BT-20, squamous cell carcinoma cell line SiHa, and transitional-cell carcinoma cell lines of the bladder T24 and RT4 were obtained from the American Type Culture Collection (Rockville, MD). To avoid interference of exogenous growth factors in growth assays (see below), all lines were weaned from fetal calf serum (FCS) and routinely grown in chemically defined culture medium W487 consisting of a mixture of four parts MCDB 202 (GIBCO, Grand Island, NY) and one part L15 media supplemented with insulin (5 μg/ml; Sigma, St. Louis, MO), transferrin (10 μg/ml; Sigma), EGF (5 ng/ml; murine; culture grade; Collaborative Research, Bedford, MA), ethanolamine (0.1 mM; Sigma), phosphoethanolamine (0.1 mM; Sigma), and hydrocortisone (0.5 μM; Sigma). Prior to growth assays cells were maintained for at least 2 weeks in W487 medium supplemented with insulin only and passaged using soybean trypsin inhibitor (Sigma). Synthetic bioactive TGF-α (residues 1-50) homologous to human TGF-α was obtained from Biotope (Seattle, WA).

Monoclonal Antibodies

Monoclonal antibody 425 has been described previously [22]. It binds to a protein determinant on the external domain of the human EGF-receptor and inhibits

binding of EGF to the EGF-receptor [10]. MAb 300-3 was generated by immunizing a Balb/c mouse with conditioned medium of a metastatic melanoma cell line (WM 266-4). This antibody binds to a 250 kDa glycoprotein secreted by melanoma cells but not expressed on the cell surface of melanoma or carcinoma cells (Herlyn et al., in preparation). Monoclonal antibodies were produced in pristane-primed, hybridoma-bearing mice and purified on Protein A-Sepharose columns (Sigma).

Growth Assay

Growth assays were performed essentially as described previously [6]. Cells were seeded in 24-well plates (Costar, Cambridge, MA) at 1.5×10^4 cells/cm^2 either in W487 medium only, W487 supplemented with 1 μM insulin, or W487 medium supplemented with 1 μM insulin and various concentrations of EGF or TGF-α as indicated. MAbs were added at various concentrations ranging between 1 and 100 nM to cultures kept in these different conditions and cell counts were determined using a Coulter cell counter at days 1, 7, and 14 after seeding. To avoid nutrient depletion, cultures were refed with media containing the appropriate ligands at days 5, 9, and 12 after seeding.

Receptor Binding Assay

Receptor binding assays were performed using human recombinant ^{125}I-EGF or ^{125}I-TGF-α. EGF (Amgen, Thousand Oaks, CA) was labeled with [^{125}I] Na using the Iodogen method as previously described [23] and TGF-α (Bachem Inc., Torrance, CA) using the chloramine T-method as described by Biswas et al. [24]. For EGF-binding assays, carcinoma cells growing in EGF-free base medium were detached with trypsin, washed with Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS, resuspended in binding buffer (phosphate-buffered saline containing 2% bovine serum albumin [Sigma]), and distributed in U-bottom tissue culture plates (Costar) at 2.5×10^5 cells/well/50 μl. Ligands diluted in binding buffer were added in 50 μl aliquots per well. Plates were incubated for 2 h at 4°C under constant motion. After incubation, cells were pelleted and washed three times with binding buffer. Cell-associated radioactivity was determined in a gamma counter. Cells and buffers were kept on ice during all steps following detachment of target cells from tissue culture plates to prevent down-regulation of the EGF-receptor. Nonspecific binding of radiolabeled EGF or MAb 425 was determined by adding excess ($>$ 100-fold) unlabeled EGF to control wells. TGF-α binding assays were performed at 4°C using confluent A 431 cell monolayers using a binding buffer consisting of Hanks' balanced salt solution containing 20 mM HEPES (pH 7.4) and 0.1% BSA. Cell associated radioactivity was determined after washing the cells three times with Hanks' balanced salt solution containing 20 mM HEPES (pH 7.4) and solubilization in 0.1 ml of 0.1 M NaOH.

Anchorage-Independent Growth Assay

Soft agar assays were done using a modification of the protocol described by DeLarco and Todaro [25]. Normal rat kidney fibroblast (NRK-49F) cells (6×10^3 cells) were suspended in 0.2 ml of DMEM containing 10% FCS and mixed with 0.6 ml of 0.5% agar noble (DIFCO, Detroit, MI) in serum-supplemented DMEM and 0.2 ml

of test sample. This mixture was placed on top of a bottom layer of 0.5% agar noble (1 ml) in DMEM supplemented with 10% FCS in 35-mm tissue culture dishes. Test samples were untreated conditioned media of carcinoma cells collected from confluent cultures between 24 and 72 h after depletion of growth factor-containing media. Plates were incubated for 10 days in a humidified atmosphere containing 5% CO_2, 95% air at 37°C, and the number of colonies was determined using an inverted light microscope. A colony was defined as a cluster of NRK-49F cells containing more than eight cells. Units of EGF equivalents/ml of conditioned medium were calculated from standard reference curves obtained using various concentrations of EGF (0, 0.156, 0.625, 2.5, 10 ng/well). TGF-α/EGF activity was assayed in the presence of 2 ng TGF-β/plate (R&D, Minneapolis, MN).

RESULTS

Inhibition of ^{125}I-TGF-α Binding to A 431 Cells

As shown in Figure 1, MAb 425 specifically competes with ^{125}I-TGF-α for binding to intact A 431 cells. Increasing concentrations of antibody (1 ng–100 μg/ml) blocked up to 90% of ^{125}I-TGF-α binding to these cells.

Expression of EGF-Receptor and Production of EGF/TGF-α-Like Mitogens by Carcinoma Cells

Table I shows the average number of EGF binding sites per cell in the tumor cell lines selected for this study as determined by specific binding of ^{125}I-EGF at saturating concentrations. Trypsinization of cells prior to receptor binding assays did not decrease binding of ^{125}I-EGF as compared to receptor binding assays performed on cells grown in monolayers (data not shown). Expression of EGF receptors ranged between 2×10^4 and approximately 1.2×10^6 sites/cell. Binding of ^{125}I-MAb 425 correlated quantitatively with surface binding of EGF (not shown).

Soft agar clonogenic assays showed secretion of bioactive EGF/TGF-α-like mitogenic activities by all carcinoma cell lines tested (Table I). Conditioned media of

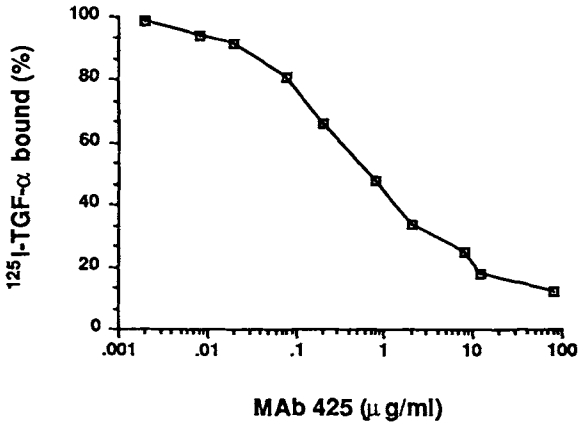

Fig. 1. Effect of MAb 425 on ^{125}I-TGF-α binding to A 431 cells. A 431 cells were incubated with 1 nM ^{125}I-TGF-α in the presence of MAb 425 and cell associated radioactivity measured as described in Materials and Methods.

TABLE I. Expression of EGF-Receptor and Production of EGF/TGF-α-Like Mitogens by Carcinoma Cell Lines

Source	Cell line	No. of EGF molecules bound per cell[a]	Mitogenic activities (ng EGF equivalents/ml) in conditioned media[b]	
			Batch 1	Batch 2
Colorectal carcinoma	SW1116	2×10^4	3.2*	2.2
	SW948	5×10^4	0.6	3.0
Bladder carcinoma	T24	3×10^5	2.8	0.1
	RT4	9×10^4	0	0.6
Pancreatic carcinoma	Capan-2	1×10^5	2.0	1.0
Breast carcinoma	BT-20	1.2×10^6	3.0	4.6
Squamous cell carcinoma	SiHa	2×10^5	0.1	0.1

[a]Binding of ^{125}I-EGF was determined at saturating conditions (40 nM). Nonspecific binding was determined in the presence of 5 μM unlabeled EGF. No. of binding sites was calculated after subtraction of nonspecific binding (< 10% of total binding).

[b]Two different batches of conditioned media were collected and tested for TGF-α-like mitogenic activity in five (batch 1) and two (batch 2) independent soft agar assays using NRK-49F cells as indicators.

*Values were calculated from dose-response curves obtained with EGF; all values different from 0 were significantly higher (Student's t-test, $r < 0.05$) than values of the negative control (unconditioned medium containing 2 ng/well TGF-β).

SW1116, SW948, Capan-2, BT-20, T24, SiHa, and RT4 contained TGF-α-like bioactivities ranging between 0.1 and 4.6 ng EGF equivalents/ml.

Growth Characteristics of Tumor Cell Lines

Cell attachment was determined one day after seeding and varied between 4×10^3 (BT-20) and 14×10^3 cells/cm^2 (T24). In W 487 medium containing insulin as the only exogenous polypeptide growth factor, cell numbers increased between 4-fold (BT-20) and 25-fold (T24) during the observation period of 7–14 days. T24 and RT4 bladder carcinoma cells proliferated vigorously and reached their plateau phase before day 14. To avoid problems with overgrowing cultures we, therefore, determined cell numbers of these cell lines in subsequent experiments at day 7 (Table II). All other cell lines continued to proliferate up to 14 days. EGF or TGF-α added to W 487 medium containing insulin further stimulated growth of the CRC cell lines SW1116 and SW948, mammary carcinoma BT-20, pancreatic carcinoma Capan-2, and squamous cell carcinoma SiHa (Table II). Growth stimulation by TGF-α was most pronounced in CRC cells SW948. Conversely, TGF-α produced insignificant increases in cell numbers of the fast proliferating bladder carcinoma lines RT4 and T24. EGF and TGF-α used at equimolar concentrations stimulated proliferation of carcinoma cells at comparable levels. In medium not containing any exogenous growth factor, CRC cells SW1116 and SW948 did not proliferate whereas all other cells grew significantly (not shown).

Inhibition of EGF/TGF-α-Dependent Carcinoma Cell Growth by MAb 425

Addition of MAb 425 to culture medium containing EGF (1 nM) inhibited effects on cell growth induced by exogenous EGF in a dose-dependent manner (Table III). EGF-induced proliferation of SW1116, Capan-2, and SiHa cells was abolished by

TABLE II. Growth Characteristics of Carcinoma Cell Lines in Chemically Defined Media Supplemented With Polypeptide Growth Factors

Source	Cell line	Cell No./cm²[a] W487 + insulin (1 μM)	W487 + insulin (1 μM) + EGF (1 nM)	W487 + insulin (1 μM) + TGF-α (1 nM)
Colorectal carcinoma	SW1116	4.0×10^4	6.0×10^4	6.8×10^4
	SW948	2.6×10^4	15.5×10^4	13.6×10^4
Pancreatic carcinoma	Capan-2	5.2×10^4	14.8×10^4	12.3×10^4
Breast carcinoma	BT-20	1.6×10^4	4.0×10^4	5.2×10^4
Squamous cell carcinoma	SiHa	17.5×10^4	24.1×10^4	21.9×10^4
Bladder carcinoma	T24	35.7×10^4	38.8×10^4	36.7×10^4
	RT4	7.2×10^4	8.6×10^4	8.0×10^4

[a]Cell Nos. were determined at day 7 (T24, RT4) or day 14 (all others) after seeding; results are expressed as average No. of cells in duplicate wells, standard deviations were < 10%.

TABLE III. Effects of MAb 425 on EGF-Mediated Carcinoma Cell Proliferation

Cell line	Percent inhibition of EGF-induced cell growth by MAb 425[a] 1 nM	32 nM	100 nM
Sw1116	50	100	
SW948	12	98	100
Capan-2	18	100	
BT-20	0	8	67
SiHa	25	100	

[a]Cells were grown for 14 days in defined medium containing 1 μM insulin, 1 nM EGF, and MAb 425 at the concentrations indicated. Inhibition of cell growth is expressed as percentage of inhibition compared to cultures incubated with control MAb 300-3.

MAb 425 at a concentration of 32 nM. Complete inhibition of EGF-induced proliferation of SW948 cells was achieved using higher concentrations of MAb 425 (100 nM), whereas EGF-dependent proliferation of BT-20 cells could only be reduced but not abolished. EGF-antagonistic effects of MAb 425 on cell proliferation were specific since control antibody 300-3 did not induce any detectable growth inhibition. Growth of the two bladder carcinoma cell lines unresponsive to exogenous EGF was not inhibited by MAb 425 at 32 nM (not shown).

Inhibition of Carcinoma Cell Growth by MAb 425 in the Absence of Exogenous EGF/TGF-α

When EGF was omitted from the culture medium, MAb 425 significantly inhibited growth of the five cell lines of CRC, pancreatic, breast, and squamous cell carcinoma origin (Table IV). Proliferation of the two bladder carcinoma cell lines was not inhibited by MAb 425. MAb 425-dependent growth inhibition was dose-dependent and ranged from 27% to 68% when compared to growth of cultures incubated with control MAb 300-3. All cells had been maintained in W 487 medium free of exogenous EGF/TGF-α for at least 2 weeks prior to growth experiments. Interference by exogenous EGF-receptor reactive ligands can therefore be excluded.

TABLE IV. Inhibition of Tumor Cell Growth by Anti-EGF-Receptor MAb 425 in Media Free of Exogenous EGF or TGF-α

	Percent inhibition of growth in the absence of exogenous EGF/TGF-α*	
Cell line	1 nM	32 nM
SW948	33	42
BT-20	28	41
Capan-2	43	64
SW1116	47	68
SiHa	27	47
T24	0	3
RT4	2	11

*Inhibition of growth expressed as percent decrease in cell Nos. compared to control wells receiving an antibody not binding to carcinoma cells (MAb 300-3) at 32 nM; cell Nos. of duplicate wells were determined at day 7 (cell lines RT4 and T24) or day 14 (all others) after seeding depending on the growth rate of the respective cell line. Inhibition of cell growth <20% was statistically insignificant (Student's t-test, $P > 0.05$).

MAb 425-induced growth inhibition was independent of insulin present in our routine culture medium since growth of BT-20, SW1116, and Capan-2 cells maintained in media free of any polypeptide growth factors was inhibited to comparable levels (Fig. 2). Growth inhibition of SW1116 cells induced by MAb 425 could be reversed by increasing concentrations of exogenous EGF (Fig. 3). When MAb 425 was removed from the culture medium after 6 days of incubation, SW1116 and SW948 cells started to proliferate at rates comparable to cultures incubated with control antibody (not shown).

DISCUSSION

We have used a panel of seven carcinoma cell lines derived from colon, pancreas, breast, and bladder tissue to address the role of EGF-receptor expression and EGF/TGF-α production in growth deregulation of carcinoma cells. All of these cell lines expressed surface EGF-receptor, as determined by specific binding of ^{125}I-EGF, and secreted detectable levels of EGF/TGF-α-like mitogens into their culture media. EGF and TGF-α added to the culture medium stimulated growth of five of the seven cell lines to comparable levels indicating that the EGF-receptor on those cells was functionally active. The bladder carcinoma cell lines T24 and RT4 showed insignificant mitogenic response to exogenous EGF/TGF-α. These results are in agreement with studies by Singletary et al. [26], who described that exogenous EGF stimulates growth of most early cultures of primary human tumor cells derived from breast, colon, and pancreas tumors and, to a lesser extent, bladder carcinoma cells.

To interrupt stimulation of carcinoma cell growth by EGF/TGF-α, we used anti-EGF-receptor MAb 425. Several lines of evidence indicate that MAb 425 acts as an EGF/TGF-α antagonist and has no intrinsic activity on EGF-receptor-mediated proliferation. As shown in this study, MAb 425 not only inhibited binding of EGF, but also of TGF-α to the EGF-receptor. We have described previously that MAb 425 blocks the EGF-dependent stimulation of growth and EGF-receptor autophosphory-

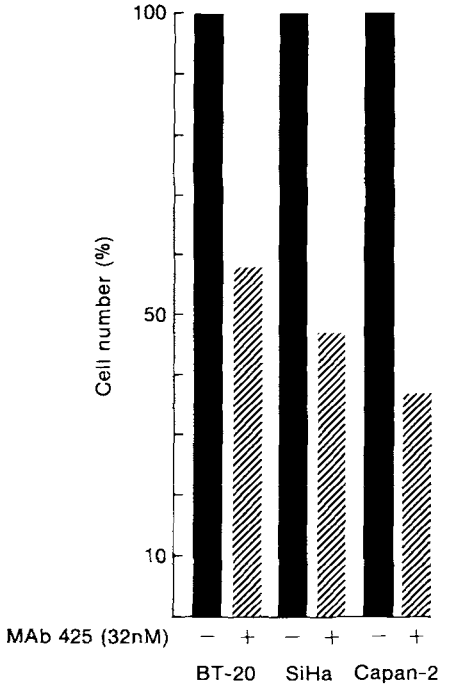

Fig. 2. MAb 425-induced growth inhibition of carcinoma cells BT-20, SiHa, and Capan-2 grown in the absence of exogenous polypeptide growth factors. Growth of control cells (full bars) maintained in the absence of MAb is shown as 100%. Growth rates of cells grown in the presence of 32 nM MAb 425 (shaded bars) is significantly reduced. Cell counts were determined 14 days after seeding.

lation in fibroblasts [10]. In addition, MAb 425 inhibited TGF-α-induced formation of inositol 1,4,5-triphosphate and increases of free cytoplasmic Ca^{++} in carcinoma cells (results unpublished). In the absence of EGF/TGF-α, MAb 425 showed no effect on these phenomena associated with activation of the surface EGF-receptor. MAb 425 induces down-regulation and internalization of the EGF-receptor in WI 38 fibroblasts [10] and SW948 colorectal carcinoma cells [27]. A fraction of internalized MAb 425 translocates to the nucleus and competes with EGF for binding to a nuclear EGF-receptor found in the chromatin of SW948 cells [27]. Translocation of MAb 425 into the nucleus was also observed in human WI 38 fibroblasts in which MAb 425 has no growth modulatory effects in the absence of exogenous EGF [10]. Based on these results, we regard MAb 425 as an inhibitor of EGF/TGF-α binding to the EGF-receptor which has no intrinsic effects on cell proliferation.

In support of this assumption, MAb 425 inhibited EGF/TGF-α-dependent proliferation of the five EGF/TGF-α responsive cell lines but had no effect on the EGF-receptor expressing bladder carcinoma cells which do not respond to EGF/TGF-α but express surface EGF-receptor. Compared to the other cell lines MAb 425 had to be used at higher concentrations (100 nM) to reduce EGF-dependent proliferation of BT20 cells. This finding may reflect overexpression of EGF-receptors on BT20 cells necessitating more MAb 425 to compete effectively with exogenous EGF for binding. To determine whether endogenous production of EGF/TGF-α contributed to the proliferation of carcinoma cells that expressed the EGF-receptor, we assessed

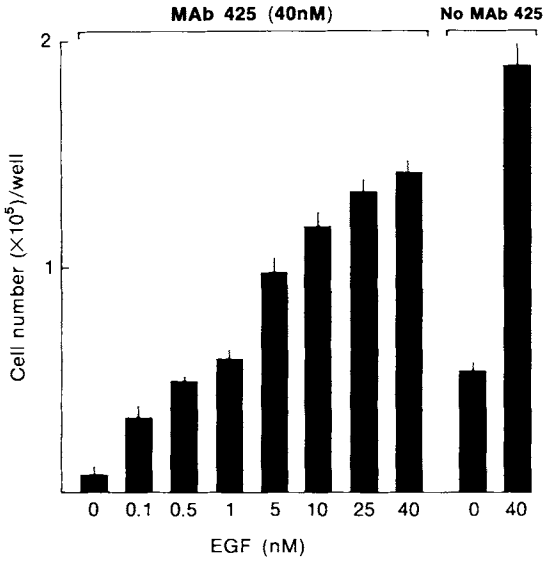

Fig. 3. Reversal of MAb 425-induced antiproliferative effects on colorectal carcinoma cell line SW 1116 by exogenous EGF. Cells were seeded in culture media containing EGF and MAb 425 at the indicated concentrations, and refed at regular intervals as described in Materials and Methods. Cell numbers were determined 14 days after seeding.

growth inhibitory effects of MAb 425 on cells adapted to grow in a chemically defined medium free of exogenous growth factors such as TGF-α or EGF, or factors contained in FCS. Under these conditions, MAb 425 significantly inhibited proliferation of all five cell lines that responded mitogenically to exogenous EGF/TGF-α but not of the two bladder carcinoma cell lines which were not stimulated by EGF/TGF-α. We found no significant difference between these cells and MAb 425-inhibited cells with regard to the level of EGF-receptor expression or secretion of EGF/TGF-α-like mitogens. These results suggest that growth autonomy of the two bladder carcinoma cell lines did not depend on endogenous TGF-α production. It remains to be determined whether other endogenously produced growth factors or the expression of oncogenes, such as H-*ras* described for T24 and other bladder carcinoma lines [28,29], can account for this finding. Alternatively, endogenously produced EGF/TGF-α may activate the EGF-receptor in these two cell lines at an intracellular location inaccessible to MAb 425. At variance with earlier results [22], we observed antiproliferative effects of MAb 425 on SW948 cells. This difference may be explained by the higher sensitivity of our current growth assays. Cell growth is monitored up to 14 days and growth-modulatory ligands are added fresh every 3 or 4 days, maintaining high concentrations throughout the incubation period. In addition, SW948 cells in the earlier study were not grown long-term in defined media. Thus, protracted effects of exogenous EGF/TGF-α could not be excluded.

Growth-inhibitory effects induced by MAb 425 were specific since MAb 425 did not inhibit growth stimulatory effects elicited by insulin or transferrin (results not shown). They were also reversible upon removal of antibody from the culture medium and could be overcome by high concentrations of exogenous EGF. No quantitative correlation could be found between the level of surface EGF-receptor expression on

trypsinized cells and growth inhibitory effects of MAb 425. This finding is interesting when compared to results obtained with NIH3T3 cells infected with retroviral vectors containing the EGF-receptor and TGF-α genes [21,30]. In this system, a high level of the EGF-receptor expression (100–200-fold as compared to control NIH3T3 cells) is required to elicit an effective mitogenic response by endogenous TGF-α. It appears that in human carcinoma cells expression of EGF-receptor at much lower levels is sufficient to mediate TGF-α-dependent mitogenic effects.

Antiproliferative effects comparable to the effects observed with MAb 425 have been described for EGF-receptor reactive MAbs 528 and 225 [31]. However, the effects of these antibodies were observed only in cell lines that overexpress the EGF-receptor and are growth-inhibited by EGF itself [32]. MAb 425, as shown in this study, inhibits proliferation of tumor cells which express EGF-receptor at lower levels and are growth-stimulated by exogenous EGF/TGF-α.

In summary, our results show that in addition to immunological anti-tumor effects described earlier [22] MAb 425 inhibits growth factor-dependent proliferation of a variety of carcinoma cells in vitro. These findings suggest that MAb 425 may have a dual therapeutic effect on EGF-receptor expressing tumor cells in vivo that have access to either autocrine or systemic EGF/TGF-α.

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REFERENCES

1. Herlyn D, Powe J, Ross AH: J Immunol 134:1300–1304, 1985.
2. Yang HM, Reisfeld RA: Proc Natl Acad Sci USA 85:1189–1193, 1988.
3. Jacobs S, Cook W, Svoboda ME, VanWyk JJ: Endocrinology 118:223–226, 1986.
4. Ohmura E, Okada M, Onoda N, Kamija Y, Murakami H, Tsushima T, Shizume K: Cancer Res 50:103–107, 1990.
5. El-Badry O, Romanus JA, Helman LJ, Cooper MJ, Rechler MM, Israel MA: J Clin Invest 84:829–839, 1989.
6. Rodeck U, Herlyn M, Menssen H-D, Furlanetto RW, Koprowski H: Int J Cancer 40:687–690, 1987.
7. Fernandez-Pol JA: J Biol Chem 260:5003–5011, 1985.
8. Schreiber AB, Lax I, Yarden Y, Eshhar Z, Schlessinger J: Proc Natl Acad Sci USA 78:7535–7539, 1981.
9. Parsons Chandler L, Chandler CE, Hosang M, Shooter EM: J Biol Chem 260:3360–3367, 1985.
10. Murthy U, Basu A, Rodeck U, Herlyn M, Ross AH, Das M: Arch Biochem Biophys 252:549–560, 1987.
11. Massague J: J Biol Chem 258:13614–13620, 1983.
12. Marquardt H, Hunkapiller MW, Hood LE, Todaro GJ: Science 223:1079–1082, 1984.
13. Todaro GJ, Fryling C, DeLarco JE: Proc Natl Acad Sci USA 77:5258–5262, 1980.
14. Carpenter G, Stoschek CM, Preston JA, DeLarco JE: Proc Natl Acad Sci USA 80:5627–5630, 1983.
15. Coffey RJ, Goustin AS, Soderquist AM, Shipley GD, Wolfshohl J, Carpenter G, Moses H: Cancer Res 47:4590–4594, 1987.
16. Hamburger AW, White CP, Dunn FE: Br J Cancer 51:9–14, 1985.
17. Hanauske AR, Buchok J, Scheithauer W, Von Hoff DD: Br J Cancer 55:57–59, 1987.
18. Imanishi K, Yamaguchi K, Suzuki M, Honda S, Yanaihara N, Abe K: Br J Cancer 59:761–765, 1989.

19. Anzano MA, Rieman D, Prichett W, Bowen-Pope DF, Greig R: Cancer Res 49:2898–2904, 1989.
20. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger W: Cancer Res 47:707–712, 1987.
21. DiMarco E, Pierce JH, Fleming TP, Kraus MH, Molloy CJ, Aaronson SA, DiFiore PP: Oncogene 4:831–838, 1989.
22. Rodeck U, Herlyn M, Herlyn D, Molthoff C, Atkinson B, Varello M, Steplewski Z, Koprowski H: Cancer Res 47:3642–3696, 1987.
23. Rakowicz-Szulczynska EM, Rodeck U, Herlyn M, Koprowski H: Proc Natl Acad Sci USA 83:3728–3732, 1986.
24. Biswas R, Basu M, Sen-Majumdar A, Das M: Biochemistry 24:3795–3802, 1985.
25. DeLarco JE, Todaro GJ: Proc Natl Acad Sci USA 75:4001–4005, 1978.
26. Singletary SE, Baker FL, Spitzer G, Tucker SL, Tomasovic B, Brock WA, Ajani JA, Kelly AM: Cancer Res 47:403–406, 1987.
27. Rakowicz-Szulczynska EM, Otwiaska D, Rodeck U, Koprowski H: Arch Biochem Biophys 268:456–464, 1989.
28. Der CJ, Krontiris TG, Cooper GM: Proc Natl Acad Sci USA 79:3637–3640, 1982.
29. Goldfarb M, Shinizu K, Perucho M, Wigler M: Nature 296:404–409, 1982.
30. DiFiore PP, Pierce JH, Kraus MH, Segatto O, King CR, Schlessinger J, Aaronson SA: Cell 51:1063–1070, 1987.
31. Masui H, Kawamoto T, Sato JD, Wolf B, Sato G, Mendelsohn J: Cancer Res 44:1002–1007, 1984.
32. Taetle R, Honeysett JM, Houston LL: JNCI 80:1053–1059, 1988.